

additional effects mediated by the polymorphic site at position 1239, immediately 5' to the stop codon, affecting translation termination. A complete and accurate picture of variation across the entire gene is required before such interactions can be studied effectively.

Richard H. Wilson,<sup>1</sup> Colin N. Moran,<sup>1</sup> John J. Cole,<sup>1</sup> Yannis P. Pitsiladis,<sup>1</sup> and Mark E.S. Bailey<sup>1,\*</sup>

<sup>1</sup>Molecular Genetics and Institute of Diet, Exercise and Lifestyle (IDEAL), Faculty of Biomedical & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK

\*Correspondence: [m.bailey@bio.gla.ac.uk](mailto:m.bailey@bio.gla.ac.uk)

### Supplemental Data

Supplemental Data include one table and one figure and can be found with this article online at <http://www.ajhg.org>.

### Acknowledgments

We are grateful to M. Sironi for unpublished data that enabled us to interpret Cagliani et al.'s Figure 1. This research was supported in part by a BBSRC research grant, no. 17/D17566.

### Web Resources

The URLs for data presented herein are as follows:

dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/index.html>

HapMap, <http://www.hapmap.org>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim/>

### Accession Numbers

The chimpanzee *ADRB2* gene sequence generated by us has been given GenBank accession number GU373723.

### References

1. Cagliani, R., Fumagalli, M., Pozzoli, U., Riva, S., Comi, G.P., Torri, F., Macchiardi, F., Bresolin, N., and Sironi, M. (2009). Diverse evolutionary histories for  $\beta$ -adrenoreceptor genes in humans. *Am. J. Hum. Genet.* 85, 64–75.
2. Hawkins, G.A., Tantisira, K., Meyers, D.A., Ampleford, E.J., Moore, W.C., Klanderman, B., Liggett, S.B., Peters, S.P., Weiss, S.T., and Bleeker, E.R. (2006). Sequence, haplotype, and association analysis of *ADRBeta2* in a multiethnic asthma case-control study. *Am. J. Respir. Crit. Care Med.* 174, 1101–1109.
3. Drysdale, C.M., McGraw, D.W., Stack, C.B., Stephens, J.C., Judson, R.S., Nandabalan, K., Arnold, K., Ruano, G., and Liggett, S.B. (2000). Complex promoter and coding region  $\beta$ 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc. Natl. Acad. Sci. USA* 97, 10483–10488.
4. Panebra, A., Schwarb, M.R., Swift, S.M., Weiss, S.T., Bleeker, E.R., Hawkins, G.A., and Liggett, S.B. (2008). Variable-length poly-C tract polymorphisms of the  $\beta$ 2-adrenergic receptor 3'-UTR alter expression and agonist regulation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294, L190–L195.
5. Moran, C.N., Yang, N., Bailey, M.E.S., Tsiokanos, A., Jamurtas, A., MacArthur, D.G., North, K., Pitsiladis, Y.P., and Wilson, R.H. (2007). Association analysis of the *ACTN3* R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *Eur. J. Hum. Genet.* 15, 88–93.

DOI 10.1016/j.ajhg.2010.01.032. ©2010 by The American Society of Human Genetics. All rights reserved.

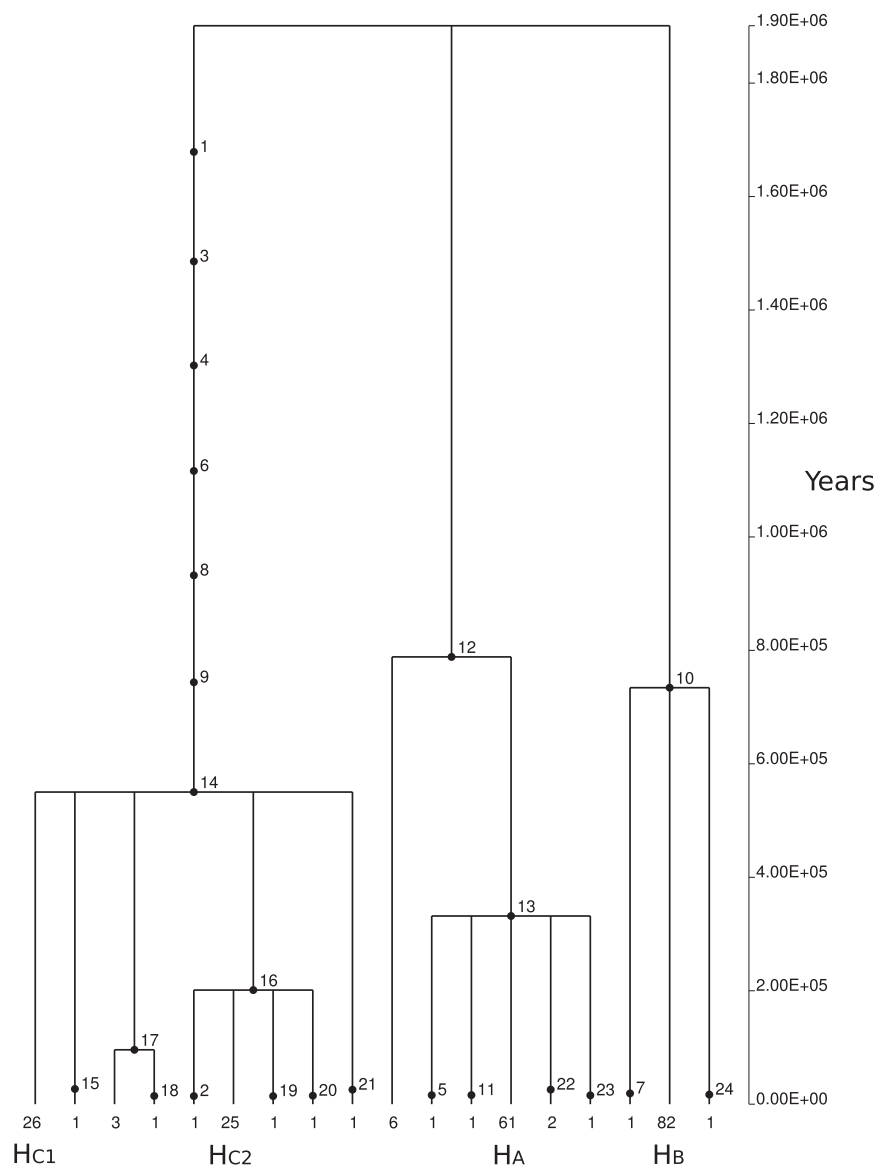
## Response to Wilson et al.

*To the Editor:* Wilson et al. noticed that the *ADRB2* (MIM 109690) haplotype network we have previously presented<sup>1</sup> displays some recurrent mutations; therefore, they propose an alternative genealogy for *ADRB2* haplotypes. Apparent homoplasies may result from different processes, namely recurrent mutation (true homoplasy) and recombination or gene conversion. It is often difficult to discriminate among these possibilities. As an example, three recurrent mutations in the *ADRB2* gene region occurred at CpG sites, suggesting that they may represent true homoplasies. Nonetheless, we agree with the authors that the network that they present might better satisfy parsimony conditions as compared to the one that we provided. We would like to mention that network analysis may not be robust in regions where recombination events are evident, especially when times to the most recent common ancestor (TMRCA) need to be estimated. For this reason, we had performed an additional analysis in our previous publication<sup>1</sup> by the use of GENETREE.<sup>2,3</sup> This program is based on a maximum-likelihood coalescent analysis and assumes

an infinite-site model so that SNPs and haplotypes that do not follow this rule have to be removed (see below).

We are grateful to Wilson et al. for noticing that the chimpanzee GenBank accession NC\_006472 contained a few sequencing errors. Specifically, a 300 bp region upstream the transcription start site of *ADRB2* displayed a relatively poor alignment to the human sequence due to the presence of several single base pair mismatches. Following Wilson et al.'s indication, we resequenced the same region in three unrelated chimpanzees, and we confirmed that the sequence in this region is identical to the one obtained by Wilson et al. and nonpolymorphic (at least in this small number of individuals). With the use of this sequence, the GENETREE estimation of the TMRCA resulted equal to 1.90 million years (standard deviation 0.53 million years) (Figure 1). As expected, this TMRCA is deeper than the one that we had previously calculated,<sup>1</sup> therefore providing further support to our previous conclusion; i.e., that *ADRB2* has been evolving under a balancing-selection regime.

With respect to the sliding window analysis presented by Wilson et al., it should be noted that the evaluation of statistical significance in multiple, nonindependent



**Figure 1. *ADRB2* Haplotype Genealogy**  
Mutations are represented as black dots and named for their physical position along gene region. The absolute frequency of each haplotype is reported, and the four major haplotypes ( $H_A$ ,  $H_B$ ,  $H_{C1}$ , and  $H_{C2}$ ) are shown.

windows poses several problems. It is not clear how and whether the authors tackled this issue. Regardless, their major finding—that an excess of polymorphism versus fixed substitution is observed at the *ADRB2* coding region—confirms our findings (we formalized this comparison in the framework of a multilocus HKA test by also taking into account population demography) and, as we stated, supports the notion whereby *ADRB2* has been subjected to balancing selection.<sup>1</sup> With respect to Wilson et al.'s sliding-window analysis, it would also be helpful to know where polymorphism data relative to the region downstream the transcript (not analyzed in our work) come from and which populations have been included in the analysis. The authors noticed a reduction of polymorphism relative to divergence in this region, but they state this might be interpreted in terms of selective sweep or SNP ascertainment bias. As for the former possibility, it is worth mentioning that a reduction in SNP occur-

rence (with no robust test for significance) is a very weak indication of a selective sweep (that would be expected to interfere with the balancing-selection regime detected in the transcribed and promoter gene region). Because no indication is provided as to how these SNPs were derived, the possibility of SNP ascertainment bias cannot be evaluated. A genomic portion largely overlapping with the 3' region that Wilson et al. analyzed has been resequenced in Yoruba and Europeans by the SeattleSNPs Variation Discovery Resource: we used these data to calculate nucleotide diversity parameters, as well as Tajima's  $D$ <sup>4</sup> and Fu and Li's  $D^*$  and  $F^*$ ,<sup>5</sup> and we found no deviation from neutrality in either population (unpublished data).

With respect to the analysis of the 3' UTR presented by Wilson et al., we confirm that we also found the polyC tract to be polymorphic in our samples. Still, because insertion/deletion polymorphisms display mutational

properties different from SNPs, we did not include them in our analyses.

Matteo Fumagalli,<sup>1,2</sup> Rachele Cagliani,<sup>1</sup> Uberto Pozzoli,<sup>1</sup> and Manuela Sironi<sup>1,\*</sup>

<sup>1</sup>Scientific Institute IRCCS E. Medea, Bioinformatic Lab, Via don L. Monza 20, 23842 Bosisio Parini (LC), Italy;

<sup>2</sup>Bioengineering Department, Politecnico di Milano, Piazza L. da Vinci, 32, 20133 Milan, Italy

\*Correspondence: [manuela.sironi@bp.lnf.it](mailto:manuela.sironi@bp.lnf.it)

## Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), [www.ncbi.nlm.nih.gov/Omim/](http://www.ncbi.nlm.nih.gov/Omim/)

SeattleSNPs Variation Discovery Resource, <http://pga.mbt.washington.edu/>

## References

1. Cagliani, R., Fumagalli, M., Pozzoli, U., Riva, S., Comi, G.P., Torri, F., Macciardi, F., Bresolin, N., and Sironi, M. (2009). Diverse evolutionary histories for beta-adrenoreceptor genes in humans. *Am. J. Hum. Genet.* 85, 64–75.
2. Griffiths, R.C., and Tavaré, S. (1994). Sampling theory for neutral alleles in a varying environment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 310, 403–410.
3. Griffiths, R.C., and Tavaré, S. (1995). Unrooted genealogical tree probabilities in the infinitely-many-sites model. *Math. Biosci.* 127, 77–98.
4. Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.
5. Fu, Y.X., and Li, W.H. (1993). Statistical tests of neutrality of mutations. *Genetics* 133, 693–709.

DOI 10.1016/j.ajhg.2010.01.032. ©2010 by The American Society of Human Genetics. All rights reserved.

## About the X-to-Y Gene Conversion Rate

*To the Editor:* It has long been accepted that productive recombination between X and Y chromosomes in humans is limited to short telomeric portions known as pseudoautosomal regions. It now appears that our picture of X-Y chromosome recombination requires revision because of the observation that gene conversion between X and Y gametologous regions occurs in humans, as reported by Rosser et al.<sup>1</sup> in the July 2009 issue of the *American Journal of Human Genetics*. The data were based on resequencing of X and Y copies of a translocation hotspot (*HSA*)<sup>2</sup> adjacent to the *PRKX* (MIM 300083) and *PRKY* (MIM 400008) genes.

In October, our group presented evidence for X-to-Y gene conversion within the *VCY* (MIM 400012) genes,<sup>3</sup> a region that lies within the male-specific portion of the Y chromosome (MSY) and is different from the *PRKY* region. Thus, both studies indicate that a new form of genetic exchange occurs between the sex-specific portions of X and Y and that this process has been active in recent human evolution.

*PRKY* and *VCY* gene conversion hotspots are similar in that both are evolutionarily conserved and lie in regions displaying a high X-Y sequence similarity (~95%); both have a block of complete X-Y identity (246 bp and 206 bp, respectively). On the other hand, the two hotspots strongly differ from each other with respect to the estimated gene conversion rate per base per generation. A conversion rate per base per generation of  $2.5 \times 10^{-6}$  to  $5.4 \times 10^{-6}$  was estimated for the *VCY* genes (a generation time of 20 years was assumed).<sup>3</sup> By contrast, Rosser et al.<sup>1</sup> obtained a range for the *PRKY* X-to-Y conversion

rate per base per generation ( $\sim 1.45 \times 10^{-4}$  to  $\sim 6.82 \times 10^{-3}$ , if a 25-year generation time is assumed) two to three orders of magnitude higher than that estimated for *VCY* and compared this rate to the rate of Y-Y gene conversion ( $2.2 \times 10^{-4}$ ) between the arms of palindromes, as reported by Rozen et al.<sup>4</sup> However, to compare analyses on different sequence lengths meaningfully, it is crucial to divide by the length of the sequence under study to get a per base rate, and Rosser et al.<sup>1</sup> don't seem to have done that. In an alternate measure of conversion rate, one can take the total length of the converted tracts and divide by the total length of the tree connecting the sequences in generations, as suggested by Rosser et al.,<sup>1</sup> but then divide by the total sequence length, as follows:  $c = 1/Lt \sum_{i=1}^n l_i$  (where  $c$  is the estimated rate of gene conversion per base per generation,  $n$  is the number of observed gene conversion events,  $l_i$  is the length in bp of the  $i$ th gene conversion event,  $L$  is the length in bp of the region under study, and  $t$  is the number of generations in the tree). By this formula, one would estimate the probability per generation per site that a site is in a gene conversion event. This recognizes that a single gene conversion event might replace a string of bases, not just one, as a substitution does; it also allows for the dependence on sequence length.

We also analyzed the *PRKY HSA* hotspot by resequencing a 2,348 bp DNA segment (chrY:7,095,919–7,098,266) (see [Web Resources](#)) in 47 Y chromosomes representing a wide coverage of the Y phylogeny and resequenced an 831 bp DNA segment (chrY:7,245,678–7,246,508) encompassing a second translocation hotspot within the *PRKY* gene (*HSB*)<sup>2</sup> in the same sample set. DNA samples came from collections of the authors, and haplogroup information is as reported.<sup>5–8</sup>

No SNPs were found in the *HSB* region. This region has been described as a weaker hotspot of translocation than